Commentary

Killing of Dendritic Cells: A Life Cut Short or a Purposeful Death?

Franca Ronchese and Ian F. Hermans

Malaghan Institute of Medical Research, Wellington School of Medicine, 6002 Wellington, New Zealand

Although we normally think of dendritic cells (DCs; references 1 and 2) as the "initiators" of immune responses and of their activity as confined largely to interaction with naive T cells in secondary lymphoid organs, there is emerging evidence that DCs are also important players in the effector phase of the immune response. Although rare, DCs form a dense network of cells in many tissues such as skin and respiratory and intestinal mucosa. This strategic location enables DCs to efficiently take up antigen and interact with effector $CD4^+$ T cells in tissues to trigger cytokine secretion and activate local immune responses. Thus, we could envisage DCs in tissues as a true sentinel system with a dual mission: to alert T cells in the lymph node and trigger local defense reactions in nonlymphoid tissues. Although immature DCs, such as those in tissues, express MHC class II mainly in intracellular compartments, local inflammation causes rapid formation of MHC class II–antigen complexes and their transport to the cell membrane, and also increases expression of costimulatory molecules (3).

Similarly to the situation for $CD4^+$ T cells, DCs can become activators of effector CTLs during $CD8^+$ T cell responses. Immature DCs, with their high phagocytic capacity, would be able to efficiently take up fragments from dying infected cells or even infectious virus and cross-present it via MHC class I (1, 2). It is important to consider that these antigen-loaded DCs would also become easy targets of activated CTLs, which require only a handful of MHC I–antigen complexes in order to activate their cytotoxic machinery. Several recently published experiments indicate that this can indeed be the case. When allogeneic DCs, or DCs pulsed with MHC class I–binding peptides, were injected into naive or immune hosts, they were rapidly eliminated by a $CD8^+$ T cell–dependent mechanism (4–7). In a more physiological situation, systemic infection with a DC-tropic strain of LCMV was shown to result in a dramatic depletion of DCs from the spleens of infected animals and development of severe immune suppression (8).

I. Hermans's present address is Human Immunology Group, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK.

Address correspondence to Franca Ronchese, Malaghan Institute of Medical Research, P.O. Box 7060, Wellington South, NZ. Phone: 64-4-389-5096; Fax: 64-4-389-5096; E-mail: fronchese@malaghan.org.nz

While the ability of DCs to act as stimulators of $CD4^+$ effector T cells is clearly advantageous to the immune response, it is less obvious what benefits to the immune response or the host would arise from CTL-mediated killing of DCs. In all cases where DCs are not virus reservoirs, their elimination would not help clear infection but would deplete the immune system of critically important cells. On the other hand, killing of DCs by CTLs could occur at a stage when further presentation of antigen is unnecessary, or could contribute to the downregulation of the immune response to prevent excessive CTL activation. In support of this latter possibility, recent reports have implicated perforin in the regulation of $CD8^+$ T cell clonal burst size. Perforin is a critical component of the CTL lytic granule, and its inactivation greatly blunts the cytotoxic activity of $CD8^+$ T cells and NK cells leading to impaired viral clearance and ineffective tumor surveillance (9). In mice, perforin deficiency is also associated with enhanced accumulation of antigen-specific $CD8^+$ T cells after viral or bacterial infection, and after DC immunization (10, 11). In humans, perforin gene defects are observed in patients with Familial Hemophagocytic Lymphohistiocytosis, a lymphoproliferative syndrome with accumulation of activated $CD8^+$ T cells (12). There is so far no direct evidence that the enhanced accumulation of activated $CD8^+$ T cells in perforin deficiencies is causally linked to DC elimination by CTLs, and other mechanisms remain possible. However, DC elimination in vivo has been found to be perforin dependent (reference 4 and unpublished data) although other studies have not confirmed this finding (7). As an additional piece of evidence in favor of the association of dysregulated T cell immune responses with enhanced DC survival, the loss of DC sensitivity to TNF-related apoptosis-inducing ligand–induced cell death has been reported in two cases of human Autoimmune Lymphoproliferative Syndrome (13). In conclusion, several lines of indirect evidence suggest that DC elimination is an important requirement for the regulation of $CD8^+$ T cell responses, and for the homeostasis of immune responses in general.

There are additional situations where killing of DCs may be an important factor in shaping the $CD8^+$ T cell immune response. In the course of viral infections, DCs presenting several viral antigens simultaneously may not be able to effi-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2001/09/F23/04 $5.00
Volume 194, Number 5, September 3, 2001 F23–F26
http://www.jem.org/cgi/content/full/194/5/F23

ciently initiate immune responses to each of them, if some of those antigens are recognized by preexisting effector CTLs, or if CTLs of one specificity develop much faster than the others. This may be the case in the well recognized phenomenon of "original antigenic sin," where an existing immune response prevents the initiation of a later, cross-reactive but independent immune response. This phenomenon has been reported long ago for antiviral antibody responses (14, 15), and more recently for antiviral $CD8^+$ T cell immune responses (16). One likely mechanism by which the inhibition could take place is through the elimination of DCs presenting novel viral antigens by the preexisting pool of cross-reactive CTLs (17). Removal of DCs presenting specific antigen also represents a potential setback in immunotherapy protocols where repeated DC administrations are used to induce and maintain CTL immune responses to tumors. Once a CTL response is established, any DCs injected to boost the response are rapidly eliminated without being able to induce further expansion of specific $CD8^+$ T cells, and with little apparent effect on the immune response (unpublished data). Thus, the DCs that can efficiently initiate a $CD8^+$ immune responses have limited restimulatory ability due to their susceptibility to CTL attack.

One paper in this issue (18) adds several new pieces of information to this picture and offers a solution to the problem of rescuing DCs from CTL killing. Medema et al. have used DC cultures and transfection experiments to convincingly demonstrate that activation of DCs by CD40L or LPS renders DCs resistant to the in vitro cytotoxic activity of CTLs. This is due to the expression in DCs of a serine protease inhibitor (SPI)-6 (PI-9 in humans), which is already known to immunologists for its ability to induce resistance to lysis in effector CTLs. SPI-6 acts by inhibiting the function of one critical component in CTL granules, granzyme B, which activates caspase-dependent DNA fragmentation causing apoptosis of target cells. As a second important observation, the authors report that resistance to CTL lysis can be induced efficiently by Th1 cells, but not by Th2 cells. This appears to be due to the combined action of Th2 cytokines, with IL-10 being one critical, but not unique, factor in this process. The overall result is that DCs that have been "helped" by antigen-specific Th1 cells become resistant to T cell lysis, while DCs "helped" by Th2 cells do not.

These results are certainly elegant and provocative but are restricted to an in vitro system. We can only speculate about the extent to which SPI-6 expression will affect DC survival and the immune response. For example, the life span of DCs in vivo is not known, and it is possible that the survival of activated DCs may be intrinsically limited. Thus, protection of activated DCs from CTL attack may not significantly extend their life span. Alternatively, DCs exposed to CTL action for an extended time in vivo may undergo eventual cell death that is not apparent in vitro due to the different time frame of the experiment. At the same time, we must recognize that tightly regulated responses such as those described by Medema et al. are likely to be activated for a purpose, and that small differences in DC survival have the potential to have considerable effects on the resulting immune responses through successive rounds of amplification. Thus, as we eagerly await the results of in vivo experiments, we can speculate on how protecting DCs from being killed may affect different stages of the T cell immune response (Fig. 1).

One first and rather obvious consequence of enhanced DC survival would be the amplification of $CD8^+$ T cell

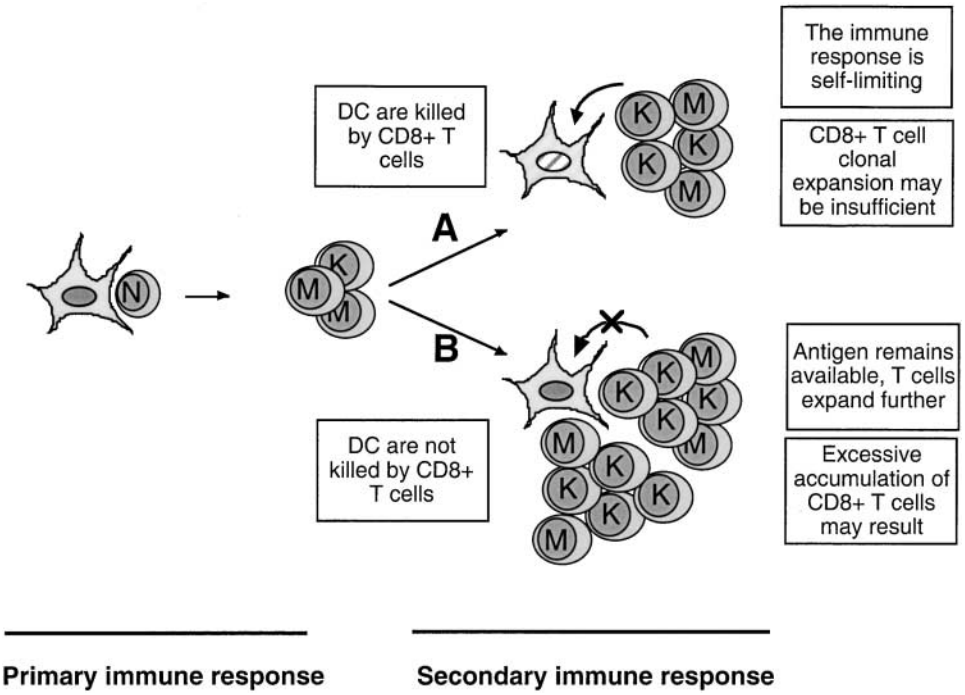

Figure 1. Killing of DCs may regulate the clonal expansion of antigen-specific $CD8^+$ T cells. Naive $CD8^+$ T cells (N) interact with antigen-presenting DCs and are induced to proliferate and differentiate into killer (K) and memory (M) progeny. (A) DCs that are susceptible to the lytic activity of CTLs are eliminated, preventing the further activation of memory cells. This may serve as a negative feedback mechanism to limit the immune response. However, the net result may also be that T cell expansion is insufficient to provide an effective response. (B) DCs that are resistant to killing by $CD8^+$ T cells will remain available for T cell recognition and induce further activation and clonal expansion of memory T cells. However, without the negative regulation provided by DC elimination, excessive accumulation of activated $CD8^+$ T cells may result. DCs may also be susceptible to killing by NK cells (not shown in Fig.). This could have an impact on both primary and secondary immune responses.

immune responses. Protecting DCs from being killed would be one additional mechanism by which T cell help can amplify CTL responses, together with the expression of costimulatory molecules through CD40–CD40L interaction, and the promotion of DC survival through CD40L and TNF-related activation-induced cytokine expression (1, 2). All these mechanisms would ensure that DCs remain available for longer to allow $CD8^+$ T cell recognition. The authors consider this possibility when they discuss the differential induction of SPI-6 by Th1 and Th2 cells. Th2 cells are not normally associated with the activation of $CD8^+$ T cells, and the observation that Th2 cells do not protect DCs from being killed could offer a mechanism for why this may be the case.

The regulation of $CD8^+$ T cell activation and clonal expansion appears to be controlled by a variety of factors. The contribution of T cell help has been long recognized as a critical parameter, and the lack of T cell help has been shown to result in abortive $CD8^+$ T cell proliferation and T cell anergy (19, 20). In contrast, the recognition of antigen in a suitable context results in the onset of a large number of cycles of proliferation, which do not require the continued presence of antigen and APCs (21, 22). Remarkably, the time of contact between naive $CD8^+$ T cells and APCs that is necessary to initiate cell division is reported as being only 2 h. After this brief contact, T cells require a further 24 to 48 h to initiate cell division. When acquisition of CTL effector function was compared with cell division using the same experimental system, both appeared to follow a similar set of rules. Again, acquisition of cytotoxic function was possible after a naive $CD8^+$ T cell had been in contact with antigen for $\sim$8 h, but a further 48–72 h were required for the development of effector function. These results would suggest that the initiation of a primary CTL immune response does not require DCs to survive for an extended period of time. Cognate interaction between T cells and DCs could easily terminate long before the T cells have acquired cytotoxic activity, or have completed clonal expansion, with apparently few long-term effects on the response. Data obtained in vivo using antigen-pulsed DCs labeled with a fluorescent tracker are consistent with those findings. Division of antigen-specific $CD8^+$ T cells is clearly apparent in the lymph node by 66–72 h. Interestingly, at approximately the same time, DCs carrying antigen disappear from the lymph node, and this is presumably due to the activation of CTL effector function (5). Therefore, premature killing of DCs would not preclude the initiation and completion of T cell clonal expansion, nor would DC resistance to CTL lysis be expected to improve it. In this regard, it is also useful to consider that MHC class I–antigen complexes at the surface of APC decay relatively rapidly, with a half- life of $\sim$5–10 h (23). Therefore, DCs are likely to lose the ability to activate naive cells well before they lose the susceptibility to CTL killing, and their longer survival is not likely to contribute to further activation of naive $CD8^+$ T cells.

In contrast to the primary immune response, resistance of DCs to CTL lysis is likely to have a much greater impact on secondary immune responses. Resistance would especially affect the survival of DCs in tissues or spleen, since effector CTLs have no significant access to the lymph node (24). Those DCs that are resistant to CTL lysis would be able to induce further clonal expansion of effector CTL – if this can occur – or of "central memory cells" in lymphoid organs. Even partial DCs resistance to CTL lysis would make a difference in these situations, as previously activated T cells only require $\sim$1 h of contact with the APCs in order to initiate cell division (25). Eventually, either through lysis by CTLs or by other mechanisms, DCs would be eliminated and the response would subside.

One puzzling observation in Medema's paper is that SPI-6 expression in DCs is induced not only by CD40 stimulation and cognate interaction with Th1 cells, but also by treatment with LPS. Why would stimuli as different as these, one being typical of the innate and one of the adaptive immune response, both elicit a similar response in DCs? Although LPS and CD40L can elicit similar responses in DCs in some respects (for example, both upregulate costimulatory molecules) their effects are not identical (IL-12 production and help to $CD8^+$ T cells are induced by CD40L, but poorly or not at all by LPS) or can even be antagonistic (LPS induces DC apoptosis in vivo, and this can be rescued by CD40L; references 1 and 2). However, both stimuli signal "true danger" and the presence of either infectious agents, or of foreign antigens that have alerted $CD4^+$ T cells. We would like to argue that one situation where DCs do need to become resistant to cell-mediated lysis is to prevent themselves being killed by NK cells. NK cells, like CTLs, induce target cell lysis by a mechanism that involves perforin, granzymes, and granule release, and that would presumably be inhibited by SPI-6. Several types of NK cells have been shown to recognize CD80 or CD86 as target antigens (26, 27), although this does not appear to be dependent on recognition through a receptor such as CD28 or CTLA-4. Recognition of CD80 or CD86 is sufficient to elicit lysis of DCs, or other activated APCs, in vitro despite their expression of high levels of MHC class I molecules. A common feature of LPS and CD40 is their ability to activate immature DCs to high expression of costimulatory molecules, thereby making them potentially more susceptible to NK cell–mediated lysis. It has not been formally shown whether lysis by NK cells is an important mechanism in the regulation of DC survival in vivo, as the above observations would suggest. If it is, it would appear critical for DCs to upregulate defense mechanisms such as SPI-6 expression in order to initiate adaptive immune responses. Indeed it has been reported that maturation decreases the susceptibility of DCs to NK cell–mediated lysis (28), despite the concomitant increase in "target" costimulatory molecules. If resistance to NK lysis is an important mechanism, other stimuli that induce upregulation of CD80 and CD86 on DCs could also be expected to activate SPI-6 expression.

Clearly, there is a lot more that needs to be known before we can decide whether killing of DCs, whether by CTLs or NK cells, is an important mechanism in regulating

in vivo immune responses, or just another trick by viruses and other infectious agents to thwart immune attack. We also need to understand to what extent these mechanisms are activated in different DC subpopulations, and how each of these DC subpopulations contributes to the regulation of immune responses. Nonetheless, the observation that DCs take the effort to protect themselves from CTL or NK cell attack suggests that killing of DCs is an important regulatory mechanism, and that DCs may remain in control of the immune response even from their deathbed.

The authors would like to thank G. Le Gros and T. Backstrom for helpful discussions, and the Cancer Society of New Zealand and Wellington Medical Research Foundation for continued support.

Submitted: 29 June 2001
Accepted: 19 July 2001

References

1. Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature*. 392:245–252.
2. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18:767–811.
3. Inaba, K., S. Turley, T. Iyoda, F. Yamaide, S. Shimoyama, C. Reis e Sousa, R.N. Germain, I. Mellman, and R.M. Steinman. 2000. The formation of immunogenic major histocompatibility complex class II-peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J. Exp. Med.* 191:927–936.
4. Loyer, V., P. Fontaine, S. Pion, F. Hetu, D.C. Roy, and C. Perreault. 1999. The in vivo fate of APCs displaying minor H antigen and/or MHC differences is regulated by CTLs specific for immunodominant class I-associated epitopes. *J. Immunol.* 163:6462–6467.
5. Hermans, I., D. Ritchie, J. Yang, J. Roberts, and F. Ronchese. 2000. $CD8^+$ T cell-dependent elimination of dendritic cells in vivo limits the induction of antitumor immunity. *J. Immunol.* 164:3095–3101.
6. Ritchie, D.S., I.F. Hermans, J.M. Lumsden, C.B. Scanga, J.M. Roberts, J.P. Yang, R.A. Kemp, and F. Ronchese. 2000. Dendritic cell elimination as an assay of cytotoxic T lymphocyte activity in vivo. *J. Immunol. Meth.* 246:109–117.
7. Ludewig, B., W.V. Bonilla, T. Dumrese, B. Odermatt, R.M. Zinkernagel, and H. Hengartner. 2001. Perforin-independent regulation of dendritic cell homeostasis by $CD8^+$ T cells in vivo: implications for adaptive immunotherapy. *Eur. J. Immunol.* 31:1772–1779.
8. Borrow, P., C.F. Evans, and M.B. Oldstone. 1995. Virus-induced immunosuppression: immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression. *J. Virol.* 69:1059–1070.
9. Kagi, D., B. Ledermann, K. Burki, P. Seiler, B. Odermatt, K.J. Olsen, E.R. Podack, R.M. Zinkernagel, and H. Hengartner. 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature*. 369:31–37.
10. Matloubian, M., M. Suresh, A. Glass, M. Galvan, K. Chow, J.K. Whitmire, C.M. Walsh, W.R. Clark, and R. Ahmed. 1999. A role for perforin in downregulating T-cell responses during chronic viral infection. *J. Virol.* 73:2527–2536.
11. Badovinac, V.P., A.R. Tvinnereim, and J.T. Harty. 2000. Regulation of antigen-specific $CD8^+$ T cell homeostasis by perforin and interferon-γ. *Science*. 290:1354–1357.
12. Stepp, S.E., R. Dufourcq-Lagelouse, F. Le Deist, S. Bhawan, S. Certain, A.P. Mathew, J.I. Henter, M. Bennett, A. Fischer, G. de Saint Basile, and V. Kumar. 1999. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science*. 286:1957–1959.
13. Wang, J., L. Zheng, A. Lobito, F.K. Chan, J. Dale, M. Sneller, X. Yao, J.M. Puck, S.E. Straus, and M.J. Lenardo. 1999. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lympho-proliferative syndrome type II. *Cell*. 98:47–58.
14. Francis, T. 1953. Influenza: the new acquayantance. *Ann. Intern. Med.* 39:203–212.
15. Fazekas de St Groth, S., and R.G. Webster. 1966. Disquisitions of original antigenic sin. I. Evidence in man. *J. Exp. Med.* 124:331–348.
16. Klenerman, P., and R.M. Zinkernagel. 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. *Nature*. 394:482–485.
17. McMichael, A.J. 1998. The original sin of killer T cells. *Nature*. 394:421–422.
18. Medema, J.P., D.H. Schuurhuis, D. Rea, J. van Tongeren, J. de Jong, S.A. Bres, S. Laban, R.E.M. Toes, M. Toebes, T.N.M. Schumacher, et al. 2001. Expression of the serpin serine protease inhibitor 6 protects dendritic cells from cytotoxic T lymphocyte-induced apoptosis: differential modulation by T helper type 1 and type 2 cells. *J. Exp. Med.* 194: 657–667.
19. Guerder, S., and P. Matzinger. 1992. A fail-safe mechanism for maintaining self-tolerance. *J. Exp. Med.* 176:553–564.
20. Kurts, C., H. Kosaka, F.R. Carbone, J.F. Miller, and W.R. Heath. 1997. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive $CD8^+$ T cells. *J. Exp. Med.* 186:239–245.
21. van Stipdonk, M.J.B., E.E. Lemmens, and S.P. Schoenberger. 2001. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat. Immunol.* 2:423–429.
22. Kaech, S.M., and R. Ahmed. 2001. Memory $CD8^+$ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat. Immunol.* 2:415–422.
23. Eberl, G., C. Widmann, and G. Corradin. 1993. The functional half-life of H-2k(D)-restricted T cell epitopes on living cells. *Eur. J. Immunol.* 26:1993–1999.
24. Sallusto, F., D. Lenig, R. Forster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 401: 708–712.
25. Iezzi, G., K. Karjalainen, and A. Lanzavecchia. 1998. The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity*. 8:89–95.
26. Chambers, B.J., M. Salcedo, and H.G. Ljunggren. 1996. Triggering of natural killer cells by the costimulatory molecule CD80 (B7-1). *Immunity*. 5:311–317.
27. Martin-Fontecha, A., E. Assarsson, E. Carbone, K. Karre, and H.G. Ljunggren. 1999. Triggering of murine NK cells by CD40 and CD86 (B7-2). *J. Immunol.* 162:5910–5916.
28. Wilson, J.L., L.C. Heffler, J. Charo, A. Scheynius, M.T. Bejarano, and H.G. Ljunggren. 1999. Targeting of human dendritic cells by autologous NK cells. *J. Immunol.* 163:6365–6370.